

EXHIBIT C

Expert Opinion

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Therapeutic options to target angiogenesis in human malignancies

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The critical role of angiogenesis in tumour growth and metastasis is now well established in the literature. Growing tumours stimulate neovascularisation through the secretion of pro-angiogenic growth factors, in particular, basic fibroblast growth factor and VEGF. Several lines of evidence have implicated VEGF in tumourigenesis, and understanding the role of VEGF in tumour angiogenesis has facilitated the development of novel targeting agents that specifically interfere with angiogenesis. Different approaches to disrupting tumour-induced angiogenesis encompass tyrosine kinase inhibitor, monoclonal antibodies, small-molecule inhibitors and transcription inhibitors. However, monoclonal antibody and tyrosine kinase inhibitors are the most advanced drug classes currently being investigated in clinical trials. So far, three anti-VEGF inhibitors, bevacizumab, sunitinib and sorafenib, have been approved for the treatment of solid human malignancies including colorectal cancer, gastrointestinal stromal tumours and renal cell carcinoma. Other antiangiogenic drugs are being investigated in various types of cancer. This review summarises the current literature on the use of these agents to interfere with VEGF, VEGF receptor, the matrix breakdown or other mechanisms involved in angiogenesis.

Keywords: angiogenesis, antiangiogenesis inhibitor, targeted therapy, VEGF

Expert Opin. Emerging Drugs (2006) 11(4):635-650

1. Background

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a fundamental process during embryonic development and is highly regulated during adult physiological events such as wound healing, ovulation and menstruation [1]. In addition to being involved in physiological functions, angiogenesis is also implicated in the pathogenesis of several diseases such as rheumatoid arthritis, psoriasis, age-related macular degeneration, proliferative retinopathies and cancer [2]. The involvement of angiogenesis in cancer biology was first postulated by Folkman in the early 1970s when he described the phenomenon of 'tumour dormancy' in the absence of neovascularisation [3]. The major implication of Folkman's postulate was that blocking angiogenesis could be a strategy for arresting tumour growth.

The hypothesis that tumour growth and metastasis were dependent on angiogenesis radically altered the field of cancer research and led to extensive research. After decades of experimental evidence, the hypothesis that tumour growth depends on angiogenesis has since been confirmed and is now widely accepted. Once a tumour is $> 2 \text{ mm}^3$ in size, its growth requires a network of blood vessels to supply both nutrients and oxygen and to remove waste products [4]. Not only does neovascularisation allow the tumour to grow from its early, smaller tumour size, it also provides a pathway for cancer cells to gain access to the systemic circulation and establish distant metastases.

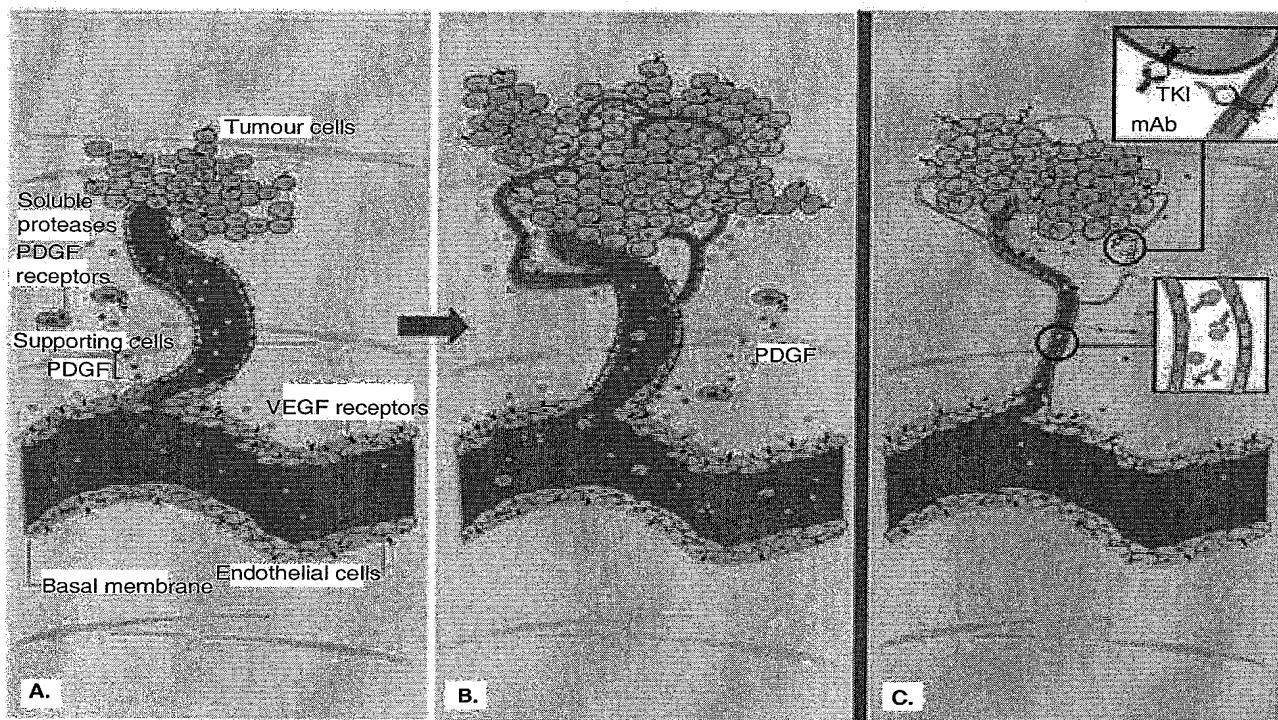


Figure 1. Schematic representation of different steps involved in tumour angiogenesis. **A.** Growing tumour and stromal tissue induce overexpression of several angiogenic factors such as VEGF, FGF and PDGF. **B.** This results in increased endothelial proliferation and migration leading ultimately to the formation of a new vascular network allowing the tumour to grow and to metastasise. **C.** Antiangiogenesis therapy using mAbs targeting VEGF (lower insert box) or VEGFR (upper insert box), as well as TKIs (upper insert box) interferes with the activation of VEGFR, and hence blocks neovascularisation.

Growing tumours stimulate the formation of new vessels through the secretion of pro-angiogenic growth factors, in particular, basic fibroblast growth factor (bFGF) and VEGF (Figure 1) [1,5]. These factors bind to receptors located on the endothelial cells composing the vessels and vasculature, leading to the secretion and activation of matrix metalloproteases (MMPs) and plasminogen activators. Activation of MMPs and plasminogen activators results in the degradation of the basement membrane, consequently allowing invasion of the surrounding matrix by the endothelial cells [6]. During migration, endothelial cells stimulated by growth factors such as bFGF and VEGF will proliferate and differentiate to form a new lumen-containing vessel. Endothelial cells form a new basement membrane and secrete additional growth factors such as platelet-derived growth factor (PDGF), which attracts supporting cells to stabilise the new vessels. Other angiogenic factors, such as angiopoietins, also participate in this process; however, their involvement is not as well characterised.

Angiogenesis is a tightly regulated process that is under the control of both pro-angiogenic and antiangiogenic factors (Figure 2). VEGFA is the most abundantly expressed angiogenic growth factor in solid and haematological malignancies [7,8]. In addition, higher levels of VEGF are observed with the progression of the disease. Finally, understanding the molecular mechanisms of VEGF in tumour angiogenesis has facilitated the development of novel agents that aim to inhibit tumour vascularisation, growth and metastasis.

2. Medical need

Cancer is the leading cause of death worldwide. Of the 5.8 million deaths worldwide in 2005, cancer accounted for 7.6 million (or 13%). In the US, cancer remains the second-leading cause of death. This year, an estimated 1.399 million Americans will learn they have cancer and 564,830 will die of it (Table 1). This estimate does not include

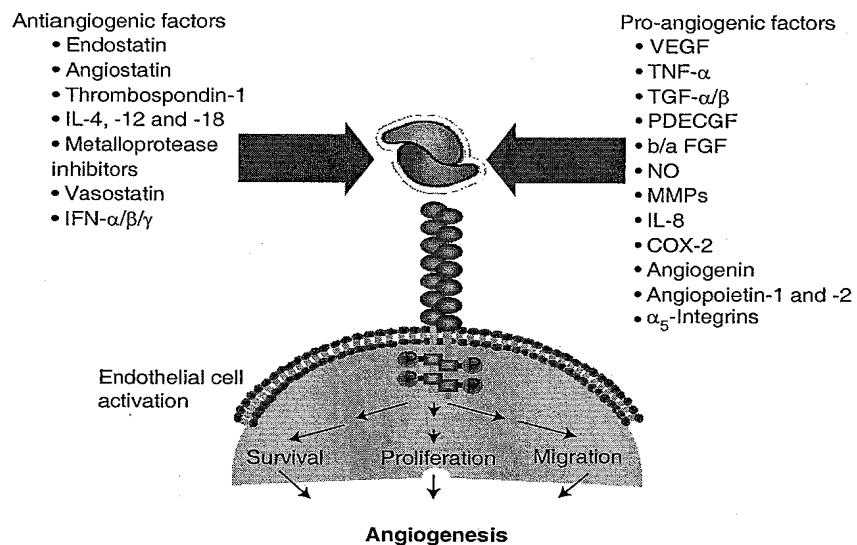


Figure 2. Pro- and antiangiogenic factors tightly regulate angiogenesis. Among the pro-angiogenic factors are VEGF, TNF- α , TGF- α/β , PDECGF, b/a FGF, NO, MMP, IL-8, COX-2, angiogenin, angiopoietin-1 and -2 and α_5 -integrins. Antiangiogenic factors include endostatin, angiostatin, thrombospondin-1, IL-4, -12 and -18, MMP inhibitors, vasostatin and IFN- $\alpha/\beta/\gamma$. b/a FGF: Basic/acidic fibroblast growth factor; MMP: Matrix metalloprotease; NO: Nitric oxide; PDECGF: Platelet-derived endothelial cell growth factor.

diagnoses of *in situ* cancer (preinvasive), except for urinary bladder cancer, or the ~ 1 million cases of nonmelanoma skin cancer that will be diagnosed this year. Although death rates from the four most common cancers – lung, breast, prostate and colorectal – continued to decline in the late 1990s, death rates for all cancers combined began to stabilise in the late 1990s, showing neither an increase nor a decrease. Furthermore, the so-called cancer population will get older as it gets larger, and by 2050, > 1.1 million people aged ≥ 75 years will be diagnosed annually. This is an increase from about 400,000 today.

In addition to surgery, the major treatment options for cancer patients are chemotherapy and radiotherapy, both of which nonspecifically inhibit normal and tumour cell proliferation. Consequently, despite undeniable improvement in the area of chemotherapy and continuous introduction of new cytotoxic agents, the management of tumours remains challenging. For instance, the general antiproliferative effects of chemotherapy and radiotherapy cause severe toxicity in normal tissue, which prohibits the use of higher therapeutic doses that might eradicate the tumours. Thus, current chemotherapeutic interventions bear significant limitations due to the occurrence of serious adverse events that compromise the quality of life of patients, and, therefore, have limited treatment outcomes. Accordingly, successful cancer treatment – ideally with a curative result across all tumour types – can only be achieved through the development and implementation of novel therapies that specifically target and inhibit signalling pathways essential for tumour development and metastasis.

3. Existing treatment

Chemotherapy alone or associated with radiotherapy remains the cornerstone of the current regimen to treat patients diagnosed with cancer. Over the years, the development and use of chemotherapy drugs has resulted in the successful curative treatment of testicular cancer, acute childhood leukaemia and Hodgkin's disease. Unfortunately, standard chemotherapy used alone or in combination with radiotherapy does not provide a curative option to the vast majority of patients with advanced cancer. In addition to limited clinical benefits, chemotherapy is also associated with severe side effects that could require a decrease in the dosage administered or even interruption of treatment. Because of the seriousness of the side effects, chemotherapy might be inappropriate in certain populations such as elderly, children and pregnant women or in patients with comorbidities. Therefore, new therapeutic options need to be developed and considered. Recently, novel therapies have emerged from a better understanding of the molecular mechanisms involved in tumourigenesis and metastasis. Specifically, intensive research has identified several potential targets to downregulate tumour angiogenesis by interfering with different agents involved in the VEGF signalling pathway. With a broader therapeutic window and less toxicity than conventional chemotherapy regimens, targeted therapy represents a promising approach to cancer treatment.

Table 1. 2006 estimated cancer deaths in the US.

Male		Female	
Cancer type	Estimated deaths	Cancer type	Estimated deaths
	291,270		273,560
Lung and bronchus	31%	Lung and bronchus	26%
Colon and rectum	10%	Breast	15%
Prostate	9%	Colon and rectum	10%
Pancreas	6%	Pancreas	6%
Leukaemia	4%	Ovary	6%
Liver and intrahepatic bile duct	4%	Leukaemia	4%
Oesophagus	4%	Non-Hodgkin's lymphoma	3%
Non-Hodgkin's lymphoma	3%	Uterine corpus	3%
Urinary bladder	3%	Multiple myeloma	2%
Kidney	3%	Brain/other nervous system	2%
All other sites	23%	All other sites	23%

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4. Current research goals

Current clinical research aims to establish therapeutic strategies that will lead to a significant increase in survival rate, considerably decrease relapses and slow down the progression of the disease. Another significant goal in clinical research is to improve quality of life for the patient. Targeted therapies have presented a promising approach for cancer treatment. However, several hurdles lie ahead that need to be tackled to provide patients with effective therapeutic strategies. It is hoped that clinical trials, along with a clearer understanding of the pathophysiology of tumorigenesis, will help establish the following goals:

- determine effective combination therapy with standard chemotherapy/radiotherapy or targeted therapies
- ascertain effective timing of first-line therapy
- establish long-term adjuvant settings

5. Scientific rationale

5.1 VEGF signalling pathway

VEGF is a diffusible, homodimeric glycoprotein that regulates vascular permeability, thereby maintaining physiological homeostasis, and stimulates angiogenesis (Figure 3) [9]. VEGF, also termed and herein interchangeable with VEGFA, belongs to the VEGF–PDGF supergene family. Other family members include VEGFB, VEGFC, VEGFD, VEGFE and placental growth factor, all showing varying degrees of homology with VEGF. VEGF and its receptors is so far the best-characterised signalling pathway in developmental angiogenesis [10–12]. VEGF expression is regulated by a number of factors including hypoxia, transforming growth factor, endothelial growth factor

or inflammatory cytokines [13,14]. VEGF actions are mediated through binding to two related receptor tyrosine kinases, VEGFR-1 (FMS-like tyrosine kinase 1; FLT-1) and VEGFR-2 (FLK-1/KDR) [12,15]. VEGFR-3 belongs to the same family of tyrosine kinase receptors; however, it binds preferentially to VEGFC and VEGFD.

Although both VEGFR-1/2 receptors are clearly implicated in different aspects of tumour angiogenesis, VEGFR-2 seems to be the major receptor by which VEGF exerts its angiogenic, mitogenic and permeability enhancing effects. The precise function of VEGFR-1 in tumour angiogenesis is more complex, and recent evidence has attributed a role to VEGFR-1 in the recruitment of monocytes [16], and haematopoietic progenitors and stem cells [17]. In addition, Hiratsuka and colleagues have shown that VEGFR-1 induces MMP-9 in lung endothelial cells and is involved in lung metastasis [18]. Recently, Kaplan and colleagues have published *in vitro* data showing that haematopoietic bone marrow progenitor cells require VEGFR-1 expression for the formation of premetastatic clusters and tumour metastasis [19]. VEGFR-1 seems to be involved in the paracrine release of growth factors from endothelial cells [20]. Preclinical studies have demonstrated that interfering with VEGF signalling, either by neutralising VEGF with antibodies or expressing a dominant-negative form of its receptor VEGFR-2 (Flk-1/KDR), inhibited the growth of tumours in rodent models [21,22]. Additional research has clearly established the crucial role of VEGFA in tumour angiogenesis, thus establishing this factor as a valuable target in cancer therapy [11,12].

5.2 VEGF as a target in cancer therapy

VEGF expression is upregulated in a majority of cancers, including haematological malignancies [23], colorectal cancer (CRC) [24], liver cancer [25], lung, thyroid, breast,

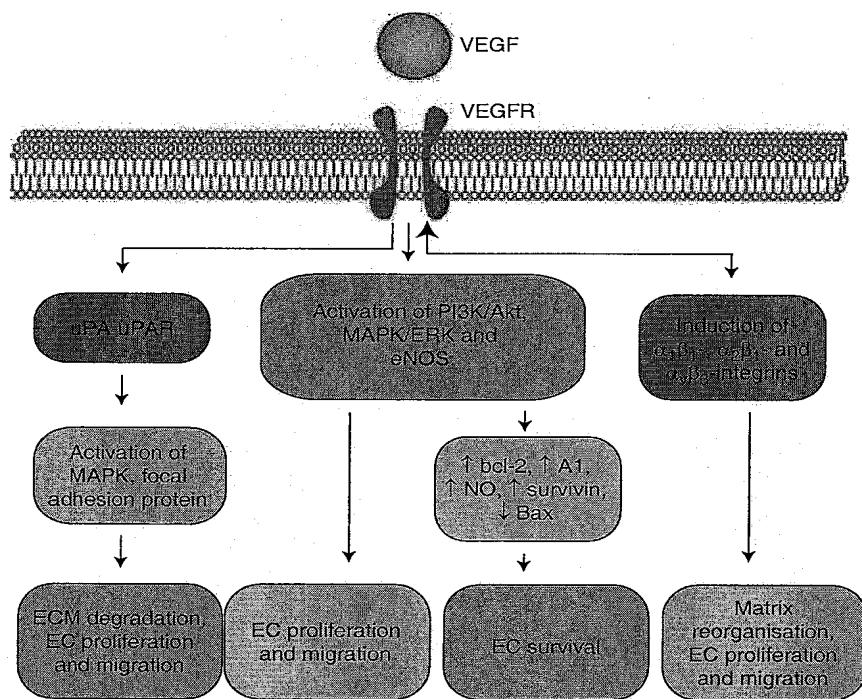


Figure 3. Simplified representation of downstream events of the VEGF/VEGFR signalling pathway. Following ligand binding, the receptor tyrosines are phosphorylated, allowing the receptor to associate with and activate a range of signalling molecules, including PI3K, MAPK or integrins. Receptor activation also induces gene expression such as the serine proteases uPA and uPAR. A cascade of intracellular signals is generated leading to cell proliferation, migration and survival.
EC: Endothelial cell; ECM: Extracellular matrix; eNOS: Endothelial nitric oxide synthetase; NO: Nitric oxide; PI3K: Phosphoinositide 3 kinase; uPA: Urokinase plasminogen activator; uPAR: uPA receptor.

gastrointestinal tract, kidney, bladder, ovary and uterine cervix carcinomas [9]. The overexpression of VEGF correlates with a more advanced stage or with a poorer prognosis in tumours of the bladder [26], breast [27], lung [28], ovary [29,30], neuroblastoma [31], renal cell carcinoma (RCC) [32] and squamous cell carcinoma of head and neck [33]. In addition to its role in solid tumours, VEGF deregulation also occurs in haematological malignancies such as acute myeloid leukaemia (AML), in which high levels of VEGF were shown to be associated with reduced survival [34]. Leukaemias express both VEGF and VEGFR-2, potentially creating an autocrine loop [35]. As well as being pro-angiogenic, VEGF induces permeability. Indeed, VEGF is detected in malignant effusions and ascites from a variety of tumour types [36,37]. *In vivo* studies showed that inhibition of VEGF blocked the formation of ascites in cells over-expressing VEGF [38]. A growing body of evidence strongly suggests that in addition to its antitumour activity benefits, targeting VEGF could also reduce its paraneoplastic secretion, leading to a potential alleviation of symptoms in the treatment of both solid and haematological malignancies.

Although VEGF/VEGFR represent the major signalling pathways in tumour angiogenesis, other signalling molecules

are involved in neovascularisation and might be of interest for the development of new therapeutic targets. Among these other molecules, PDGF-B/PDGF receptor (R)- β was reported to play an important role in the recruitment of pericytes and maturation of the microvasculature in PDGFR-deficient mice [39,40]. Additional *in vivo* studies have demonstrated that interfering with the PDGFR signalling pathway led to the inhibition of metastasis and tumour growth in several tumour models [41].

Angiopoietins are involved in the remodelling, maturation and stabilisation of the vascular network. They are a family of extracellular ligands that recognise and bind to Tie2, an endothelial cell-specific receptor tyrosine kinase (reviewed by Tait and Jones) [42]. Interestingly, although sharing genomic similarities, the angiopoietins can induce different responses in binding to the Tie2 receptor. Angiopoietin-1 (Ang-1) acts as a maturation factor promoting pericytes and smooth cell recruitment necessary to the developing vessel. Ang-2, however, acts as a destabilising signal observed prior to vessel sprouting or regression. Thus, Ang-1 and -2 play critical, but different, roles in angiogenesis. Ang-2 is mostly expressed in human tumour-induced neovasculature, and hence represents an

Table 2. Pharmacokinetic data of tyrosine kinase inhibitors.

Drug	IC ₅₀					Reference
	VEGFR-1	VEGFR-2	VEGFR-3	PDGFR	c-KIT	
AEE-788	59	77	33	320	79	[102]
AMG-706	2	3	6	84	8	[85]
AZD-2171	5	< 1	< 3	5	2	[129]
CEP-7055	74	18	8	252	ND	[105]
CP-547/632	ND	11	ND	2820	ND	[99]
PTK-787	77	37	660	580	730	[79]
Sorafenib	ND	90	20	57	68	[130]
Sunitinib	ND	10	ND	10	1 – 10	[131]
ZD-6474	1600	40	110	ND	ND	[90]

Disclaimer: data are not comparable and should be evaluated individually.

ND: No data; PDGFR: Platelet-derived growth factor receptor; VEGFR: VEGF receptor.

attractive candidate target for antiangiogenic cancer therapy. In mice harbouring human tumours, the selective inhibition of Ang-2 resulted in reduced endothelial cell proliferation, which is consistent with an antiangiogenic therapeutic mechanism [43]. Recent data have showed that tumour-bearing mice treated with Ang-4 inhibitors decreased interstitial fluid pressure, migration and growth factor-induced angiogenesis [44]. As studies unravel the role of angiopoietins in angiogenesis, these molecules represent a potential therapeutic target.

6. Competitive environment

Different approaches to disrupting tumour angiogenesis include tyrosine kinase inhibitors (TKIs), monoclonal antibodies, small molecule inhibitors and transcription inhibitors. A list of current TKIs used in clinical trials and their pharmacokinetics data are listed in Table 2. This review summarises the current literature on the use of these molecules to interfere with VEGF, VEGFR, matrix breakdown and other mechanisms involved in angiogenesis.

6.1 Targeted therapies interfering with VEGF

6.1.1 Bevacizumab

Bevacizumab is a humanised monoclonal antibody against VEGF. In February 2004, the FDA approved bevacizumab for first-line therapy of CRC in combination with an IFL-based (irinotecan, fluorouracil and leucovorin) regimen. In CRC, a randomised multi-centre study evaluating the clinical benefits of bevacizumab combined with a bolus IFL in first-line treatment of CRC showed an advantage in median duration of overall survival, median duration of progression-free survival (PFS), response rate (RR) and median duration of response [45]. The most significant clinical toxicities reported in bevacizumab-treated patients were thrombosis, hypertension, proteinuria and bleeding. In addition, gastrointestinal perforation (1.5% of those treated with bevacizumab), as well as an increase

in diarrhoea, leukopenia and hypertension were also described. Results from the Three Regimens of Eloxatin Evaluation (TREE)-2 trial study in patients with metastatic CRC presented at the 2005 American Society of Clinical Oncology (ASCO) meeting showed that the addition of bevacizumab to an oxaliplatin/fluorouracil regimen in first-line therapy was well tolerated with no unexpected toxicity [46]. Several other trials studying the clinical benefits of bevacizumab combined with different anti-EGFR agents, such as erlotinib, panitumumab or cetuximab are ongoing.

In metastatic RCC (mRCC) refractory to immunotherapy, patients treated with bevacizumab experienced a significant prolongation of time to progression (TTP) compared with the placebo group [47]. The most common side effects reported are hypertension and asymptomatic proteinuria ($n = 116$). In a double-blind Phase II trial comparing bevacizumab plus erlotinib versus bevacizumab plus placebo, data showed that the combination was safe and well tolerated, although adding erlotinib did not improve efficacy [48]. However, PFS of 8.5 months observed in patients treated with bevacizumab appears to be more favourable than what is reported with the use of IFN- α , thus suggesting a potential clinical benefit for bevacizumab in RCC. Given the promising activity of bevacizumab, several clinical trials investigating the clinical advantages of bevacizumab with IFN- α_{2b} therapy (CALGB 90206; $n = 600$) [49] or in addition to erlotinib plus imatinib are currently in progress [50].

The IFN- α carboplatin/paclitaxel regimen caused a higher RR (31.5% in the high-dose arm versus 18.8%) and longer median TTP (7.4 months in the high-dose arm versus 4.2 months) than chemotherapy alone [51]. Bevacizumab was generally well tolerated; however, severe haemoptysis episodes among patients with squamous cell histology were observed. Another study examined the clinical benefits of bevacizumab with the carboplatin/paclitaxel regimen as first-line therapy in 842 patients with nonsquamous non-small cell lung cancer

(NSCLC). This US cooperative group Phase III trial (E4599) reported a higher RR, longer PFS and increased survival in the bevacizumab/chemotherapy arm compared with the chemotherapy-alone arm [52]. In a trial combining bevacizumab with erlotinib in patients with recurrent NSCLC, preliminary data showed antitumour activity, with partial response achieved in 20% of patients and stable disease in 65% [53]. The most common adverse events reported ranged from mild-to-moderate and included rash, diarrhoea and proteinuria. Bevacizumab in combination with erlotinib continues to be investigated in recurrent or refractory NSCLC [54].

A Phase II metastatic pancreatic cancer study assessed the combination of bevacizumab plus gemcitabine in 52 patients with stage IV pancreatic cancer [55]. The data revealed promising therapeutic activity with an objective RR of 21%, a median PFS of 5.4 months and a 6-month overall survival (OS) of 77% reported. Adverse events included hypertension, thrombosis and bleeding episodes. The promising efficacy warranted two further ongoing trials: the European Phase III trial (BO17706) and the US Cooperative Group Phase III trial (CALGB 80303). Both trials will investigate the therapeutic benefits of bevacizumab when added to gemcitabine alone or with erlotinib. Zhu and colleagues recently reported encouraging results from a Phase II study investigating the therapeutic benefits of bevacizumab combined with a gemcitabine and oxaliplatin regimen in patients with advanced hepatocellular carcinoma [56]. Data demonstrated that this combination was generally well tolerated, with the most common grade 3–4 adverse events being fatigue, transient elevation of transaminases, nausea/vomiting and hypertension. Although no patients achieved a complete response, the overall response rate was 20% and the rate of PFS at 6 months 48%.

In a Phase III metastatic breast cancer (mBC) trial ($n = 462$), the addition of bevacizumab to capecitabine in a cohort of taxanes-and-anthracycline-refractory patients failed to show an improvement in PFS and OS [57]. Bevacizumab is also under investigation. The addition of bevacizumab to a carboplatin/albumin-bound form of paclitaxel and trastuzumab regimen showed promising antitumour activity among patients with mBC [58]. Of nine evaluable patients, eight achieved a major clinical response. This combination regimen is being investigated further in the neoadjuvant setting. The clinical activity of bevacizumab alone or in combination is being examined in ovarian cancer, recurrent cervical cancer, hormone-refractory prostate cancer, refractory or relapsed AML and malignant melanoma, as well as head and neck cancer. Bevacizumab is also being explored as neoadjuvant treatment.

6.1.2 HuMV833

HuMV833 is a humanised monoclonal antibody that recognises two of the principal VEGF isoforms, VEGF₁₂₁ and VEGF₁₆₅. Results from a Phase I trial showed that HuMV833 was safe and well tolerated in patients with solid tumours [59]. Infusions were well tolerated and there were no grade 3 or 4 toxicities related to the antibody reported. The most common

adverse events included fatigue/asthenia, nausea, vomiting, gastrointestinal symptoms and rash. Recently, clinical trials of HuMV833 have begun in Europe.

6.1.3 VEGF-Trap R1R2

VEGF-Trap R1R2 is a derivative of the soluble form of VEGFR-1, which irreversibly binds to VEGF. This VEGF blocker is a chimeric fusion molecule composed of the second immunoglobulin domain of VEGFR-1 and combined with the third immunoglobulin domain of VEGFR-2 [60]. VEGF-Trap R1R2 was engineered to minimise interactions with the extracellular matrix, but still retains its potent affinity for VEGFR-2 [60]. Preclinical studies showed it inhibits tumour growth in various models [61,62]. In a Phase I study, VEGF-Trap administered to patients with solid tumours did not induce antibody response with evidence of biological activity [63]. At present, a Phase I clinical trial is investigating the side effects of VEGF-Trap R1R2 in patients with relapsed or refractory advanced solid tumours or non-Hodgkin's lymphoma.

6.2 Targeted therapies interfering with VEGF receptor

6.2.1 IMC-1C11

IMC-1C11 is a chimeric monoclonal antibody against VEGFR-2, which is located on tumour-associated capillary blood vessels [64]. IMC-1C11 blocks the binding of the ligand to the receptor and subsequently inhibits downstream events such as VEGFR and MAPK activation. This agent is presently being examined in a Phase I trial in patients with liver metastases from CRC carcinoma [65]. IMC-1C11 appears to be safe and well tolerated, although 7 out of 14 patients experienced detectable levels of antibodies against IMC-1C11. A fully human anti-VEGFR-2 has been produced as a second-generation agent to be less immunogenic for chronic administration as monotherapy or in combination with chemotherapy or radiotherapy.

6.2.2 Sunitinib malate

Sunitinib is an oral small-molecule TKI against haematopoietic class 3 tyrosine kinase receptors (FLT-3), stem cell factor receptors (c-KIT), VEGFR and PDGFRs [66,67]. TKIs compete with the ATP binding site of the catalytic domain of several oncogenic tyrosine kinases and inhibit their phosphorylation, thus preventing the activation of the intracellular signalling cascade. A Phase I study investigating the biological activity of sunitinib in patients with AML showed that the phosphorylation of FLT-3 was inhibited in 50% of patients with wild-type FLT-3 and in 100% of patients with mutated FLT-3 [68]. The majority of gastrointestinal stromal tumours (GIST) harbour mutations in the receptor tyrosine kinase KIT or PDGFR-A and are responsive to imatinib. Unfortunately, tumours develop resistance often due to amino acid mutations in the kinase domain of the targeted receptor, thus preventing or weakening the interaction with the inhibitor. *In vitro* studies have demonstrated that sunitinib potently inhibited various imatinib-resistant KIT variants [69,70].

The therapeutic advantages of sunitinib were examined as second-line therapy in patients with RCC. RCC is one of the most resistant tumour types in oncology, and, so far, cytokines are the only agents that have been shown to induce tumour regression in some patients with RCC. A Phase II clinical trial conducted in 63 patients showed promising activity, with partial response observed in 25 patients and stable disease achieved in 17 patients [71].

Data from a Phase III randomised clinical trial investigating the clinical benefits of sunitinib as first-line therapy in patients with mRCC demonstrated a statistically significant improvement in PFS and objective response rate for sunitinib over IFN- α [72]. Sunitinib was also investigated in an open-label, single-arm clinical trial as second-line treatment of mRCC that had progressed despite previous cytokine therapy [73]. The results of this study revealed a partial response of 34% and a median PFS of 8.3 months. Thus, sunitinib demonstrated therapeutic benefits in mRCC with a manageable adverse events profile. This agent was also investigated in a Phase III trial in GIST patients who failed imatinib therapy. An interim analysis showed a significant increase in TTP as well as OS in the group treated with sunitinib [74]. Clinical benefits of sunitinib are also being investigated in NSCLC and preliminary data have shown that stable disease and partial response have been achieved [75]. Adverse events were generally mild-to-moderate and included fatigue, diarrhoea and nausea. In January 2006, sunitinib was approved by the FDA for imatinib-resistant and imatinib-intolerant GIST and for RCC.

6.2.3 Sorafenib

Sorafenib is an oral TKI against VEGFR-2, VEGFR-3, PDGFR, FLT-3, c-KIT, c-Raf1 and B-Raf [76]. A Phase I trial investigated the clinical benefits of sorafenib with doxorubicin in patients with primary hepatic cancer. Study data showed that sorafenib was well tolerated. The most common adverse events reported were hand-foot syndrome, diarrhoea and hypomagnesaemia [76]. Data from a Phase II trial of sorafenib in patients with mRCC demonstrated significant disease-stabilising activity in the arm receiving the active drug compared with placebo group results [77]. The most common drug-related adverse effects in all 202 patients with RCC were rash, hand-foot syndrome and fatigue [78]. Grade 3/4 drug-related events occurred in 47% of patients, with the most common being hypertension (24%), hand-foot syndrome (13%) and fatigue (5%). A large number of ongoing clinical trials evaluating the therapeutic advantages of sorafenib alone or in combination with chemotherapy are ongoing. In December 2005, the FDA approved sorafenib for advanced RCC.

6.2.4 PTK-787/ZK-222584

PTK-787 is an oral TKI of all VEGFRs with a higher potency toward VEGFR-2 [79]. Other receptors targeted by this agent include PDGFR and c-KIT. Preclinical studies demonstrated

antitumour activity against a broad range of tumours including colorectal, prostate, renal, hepatocellular and myeloma. Clinical studies of this agent against various tumour types yielded mixed results. A Phase III trial of PTK-787 in combination with FOLFOX4 in metastatic CRC as first-line failed to reach statistical significance for PFS [80]. A Phase I clinical trial evaluating the safety and efficacy of PTK-787/ZK-222584 in patients with liver metastases from solid tumours, showed a safe toxicity profile and promising activity [81]. A report of preliminary results from a Phase II clinical trial investigating the therapeutic benefits of PTK-787/ZK-222584 as a single second-line agent for NSCLC showed that disease control was achieved in 58% of patients [82]. Other clinical trials investigating PTK-787/ZK-222584 in metastatic neuroendocrine tumours and imatinib mesilate-resistant metastatic GIST are currently ongoing [83,84]. The most frequently reported adverse events associated with PTK-787 were nausea, fatigue, vomiting and dizziness [81]. All were considered reversible with drug discontinuation. In addition, several Phase I/II trials are investigating the clinical benefits of PTK-787 alone or in combination with chemotherapy in various tumours such as CRC, recurrent glioblastoma multiform, ovarian or prostate cancer.

6.2.5 AMG-706

AMG-706 is a potent oral TKI against VEGF, PDGF and c-KIT receptors [85]. It has demonstrated antiangiogenic and antitumour activity. Data from a Phase I study presented at the 2005 ASCO meeting showed that AMG-706 in patients with advanced solid tumours was safe at doses up to 125 mg/day [86,87]. Preliminary data were promising, as they indicated vascular changes and stable disease in the vast majority of patients. Most frequently reported adverse events were hypertension, fatigue, diarrhoea, headache and nausea. AMG-706 is currently undergoing several Phase I/II clinical trials alone or in combination with chemotherapy in CRC, GIST, NSCLC and thyroid cancer. Among these studies, a Phase I clinical trial evaluating the safety and clinical activity of AMG-706 in thyroid cancer showed that this agent was well tolerated and objective response was achieved in 43% of patients [88]. AMG-706 is also being investigated in combination with panitumumab (anti-EGFR monoclonal antibody) in NSCLC, and preliminary data revealed that this regimen was safe and exhibited clinical activity [89].

6.2.6 ZD-6474 and AZD-2171

These two agents are oral small TKIs targeting the VEGFR pathway [90]. ZD-6474 was also found to have additional inhibitory activity against EGFR [91] and oncogenic RET kinases [92]. Preclinical studies have yielded data consistent with a potent inhibition of the VEGF signalling pathway, suggesting potential use in a broad range of tumours including colon, lung, prostate, breast and ovarian cancers [90]. Both agents are being investigated in various cancer types. In a Phase II trial examining the clinical benefits of ZD-6474 in

patients with previously treated mBC, ZD-6474 was well tolerated, but showed limited monotherapy activity [93]. The lack of objective response in this cohort of patients might be due to the advanced stage of disease in this heavily pretreated population. Data from another Phase II clinical trial investigating the efficacy and safety of ZD-6474 compared with that of gefitinib in NSCLC demonstrated a significant prolongation of PFS among the group receiving ZD-6474 [94]. ZD-6474 is currently being investigated in hereditary metastatic medullary thyroid cancer, and preliminary data yielded clinical activity warranting further study [95]. The most common drug-related adverse events included diarrhoea, rash, nausea, hypertension, fatigue, anorexia, acneiform rash and maculopapular rash [96]. Yet another Phase II trial investigated ZD-6474 in combination with docetaxel, demonstrating that the addition of ZD-6474 prolonged PFS and warranted a Phase III trial [97].

6.2.7 Other TKIs

GW-786034 is another oral TKI with activity against VEGFR that has demonstrated antitumour and antiangiogenic activity *in vitro* and *in vivo* [98]. It is currently undergoing Phase II clinical trials on refractory multiple myeloma, soft tissue sarcoma, ovarian cancer and RCC. CP-547,632 has shown antitumour and antiangiogenic activity against VEGFR-2 in several preclinical models [99]. This agent is currently in a Phase II clinical trial for recurrent or persistent small-volume ovarian cancer. Another TKI, AG-013736 is an oral receptor TKI of VEGFR, PDGFR- β and c-KIT [100]. Evaluated in a Phase I clinical trial among patients with advanced solid tumours, AG-013736 demonstrated clinical activity in RCC, adenoid cystic cancer and lung cancer. Dose-limiting toxicities included hypertension, haemoptysis and stomatitis. These are manageable with appropriate medication or dose reduction. In a Phase II study in patients with cytokine-refractory RCC, AG-013736 demonstrated clinical activity, the best response being a partial response of 40%. Within a median follow-up of 1 year, 69% of patients remained in the study with response or stable disease [101]. This agent is undergoing a Phase II clinical trial as monotherapy in thyroid cancer and in combination with gemcitabine in pancreatic cancer. The agent AEE-788 is a dual family EGFR/ErbB2 and VEGFR TKI [102]. Preclinical data showed antitumour activity in various models [103,104]. Finally, CEP-7055 is a novel orally active inhibitor of all three VEGFR kinases with broad preclinical antitumour and antiangiogenic activity [105], now entering a Phase I clinical study.

6.2.8 RPI-4610

RPI-4610, part of a new class of drug termed chemically stabilised ribozymes, is synthesised to target, bind and cleave a specific mRNA sequence. RPI-4610, administered intravenously, targets the mRNA for VEGFR-1 [106]. A Phase I clinical trial in patients with refractory solid tumours showed grade 1/2 infusion reactions as the most

common toxicities [107]. A Phase II clinical trial is currently investigating the effectiveness of RPI-4610 in patients with metastatic kidney cancer.

6.3 Targeted therapies interfering with matrix breakdown

The involvement of proteolytic activity in angiogenesis is well established. The extracellular matrix (ECM) surrounding endothelial cells must be broken down to allow cell migration and proliferation. MMPs belong to a family of proteolytic enzymes that degrade every component of the ECM, and in doing so, contribute to the angiogenic process. There is evidence that aberrant MMP expression contributes to the invasive growth and spread of a variety of solid malignancies including gastric cancer [108,109]. Controlling the activity of MMPs through inhibitors has been considered a potential target for tumour therapy, but clinical trials yielded disappointing results.

Incyclinide (COL-3), an oral MMP inhibitor (MMPI), showed biological activity in AIDS-related Kaposi's sarcoma [110]. The most common adverse events were photosensitivity and rash. BMS-275291, a broad spectrum MMPI, failed to achieve partial or complete tumour responses in patients with advanced or metastatic cancer [111]. The most frequently reported adverse events were joint toxicity, rash, fatigue, headache and nausea. In a recent study evaluating two different doses, this agent demonstrated limited clinical activity in hormone-refractory prostate cancer with bone metastases [112]. Other MMPIs, such as BAY-129566 or BB-2516, have failed to show therapeutic activity in human malignancies despite preclinical antimetastatic and antiangiogenic activity.

The reasons for the disappointing results observed with MMPIs in cancer therapy remain unclear. The negative results of MMPIs reported in several studies have definitely raised serious concerns, such as whether or not to pursue the evaluation of MMPIs as a therapeutic strategy in oncology.

6.4 Targeted therapies interfering directly with angiogenesis

Thrombospondin-1 (TSP-1) is a naturally occurring inhibitor of angiogenesis that limits vessel density in normal tissues and curtails tumour growth. ABT-510, a promising new agent, is a TSP-1 analogue. A Phase I study in patients with advanced solid malignancies showed a favourable toxicity profile, with the most common toxicities observed being injection-site reactions and fatigue. Stability of disease was observed in a significant number of patients, warranting further clinical trials [113]. A Phase II trial testing the clinical benefits of ABT-510 in head and neck cancer is currently underway.

An additional avenue being explored is integrin protein, which plays an essential role in cell-cell and cell-matrix adhesion. Integrins are cell surface adhesion molecules coupling the extracellular environment to the cytoskeleton, as well as receptors for transmitting signals important for cell migration, invasion, proliferation and survival. Deregulation of adhesion can lead to

pathological processes, including tumour metastasis, either by disrupting the normal anchorage, thereby altering cell movement and regulatory signalling, or by promoting inappropriate adhesion. One member of the integrin family, $\alpha_v\beta_3$ -integrin, is expressed in tumour cells as well as the new vasculature of various tumours. Supporting evidence for the integrin involvement in tumour angiogenesis was recently reported by Nikolopoulos and colleagues who demonstrated that the β_4 subunit of integrin promotes endothelial migration and invasion [114]. Therefore, agents that target integrins are currently being evaluated as potential therapeutic options to treat tumours. Such agents include abegrin, a monoclonal antibody, and cilengitide (EMD-121974), a cyclic peptide. Both interfere with the $\alpha_v\beta_3$ -integrin and are under investigation in Phase I/II studies.

Another antiangiogenic drug used in human malignancies is thalidomide, although its exact mechanism of action is still unclear. Thalidomide was once notorious for producing severe deformities in the arms and legs of newborn babies whose mothers were given the drug during pregnancy. Off the market for decades, it has recently emerged as a somewhat effective treatment for several cancers. Thalidomide and its immunomodulatory analogues are being investigated in several Phase II/III trials for treating various tumours including multiple myeloma, RCC, prostate cancer and hepatocellular cancer [115].

Finally, endostatin, an endogenous angiogenesis inhibitor, represents an additional target for cancer therapy (reviewed in [116]). Clinical trials evaluating the safety of recombinant human endostatin in patients with advanced solid tumours showed that this agent was safe and well tolerated, having minor antitumour activity [117].

6.5 Developmental issues

The extensive research in the field of angiogenesis has remarkably helped the scientific community to decipher the pathophysiology of tumour angiogenesis. Fundamental aspects of this highly complex phenomenon have been identified and led to the emergence of several targeted agents. Targeted therapies have radically altered the traditional therapeutic algorithm used in the clinic and have challenged the way clinical trials are organised. These novel treatment options have shown promising activity and brought hope to the patient community; however, several challenges still lie ahead.

6.5.1 The need for combination therapies

Tumour angiogenesis is a highly complex process involving multiple signalling pathways. Although this review focuses on the role of angiogenesis in tumour growth, other intracellular mechanisms such as the epithelial growth factor receptor signalling pathways are also involved (reviewed in [118]). Based on current evidence, it seems clear that, with a few exceptions, effective therapy will probably rely on a multidisciplined approach rather than a single therapeutic tactic. Several studies have shown that combining antiangiogenic agents with chemotherapy or radiotherapy resulted in additive or synergistic antitumour activity. The mechanism responsible for

this potentiation is still unclear, and two hypotheses have been proposed to explain the synergistic therapeutic effects observed in solid tumours. One hypothesis is that antiangiogenic therapy may normalise the high interstitial pressure in tumours, resulting in improved oxygenation, better blood perfusion and, consequently, higher delivery of chemotherapeutic drugs [119]. An alternative hypothesis suggests that chemotherapy delivered at close, regular intervals with no prolonged drug-free break periods preferentially damages endothelial cells in tumour blood vessels [120,121]. This regimen, also called metronomic dosing, sustains antiangiogenic activity and presents an improvement over other regimens using maximum-tolerated dose that require extending drug-free periods to allow patients to recover from associated toxicity. In addition, because these cells are rapidly dividing and might require VEGFA to survive, the simultaneous use of an antiangiogenic agent with standard chemotherapy may intensify the cellular death process. This is a step towards personalised medicine.

A substantial challenge that lies ahead of us is to establish the most effective combination of antiangiogenic agents, other targeted therapies and traditional therapies to improve clinical outcomes. Two strategies may be followed: a vertical approach whereby multiple targets (ligand, receptor, downstream factors) within the same signalling pathway are being inhibited; or a horizontal approach targeting simultaneously multiple signalling pathways to prevent the generation of escape variants, a strategy routinely utilised with polychemotherapy. Several clinical trials are evaluating the combination of various angiogenesis inhibitors with other targeted therapies such as EGFR or HER2 inhibitors. A Phase I/II trial investigating the clinical benefits of bevacizumab with erlotinib (HER1 inhibitor) in patients with recurrent NSCLC showed that this combination was well tolerated and active [53]. Of 40 patients enrolled in this study, 8 achieved partial response and 26 had stable disease, for a clinical benefit of 85%. Interim results of another combination therapy from a Phase II trial evaluating the efficacy and safety of bevacizumab and cetuximab with or without irinotecan (Bowel Oncology and Cetuximab Antibody [BOND]-II) in patients with irinotecan-refractory CRC demonstrated a tolerable safety profile and favourable clinical activity [122]. Further trials, including bevacizumab combined with sorafenib, bevacizumab combined with panitumumab (monoclonal antibody against EGFR) or bevacizumab combined with ABT-510, are currently being conducted.

6.5.2 The need to establish biological markers

There are still some difficulties associated with the clinical evaluation of the efficacy of these drugs due to the lack of reliable markers (Figure 4) [123]. Unlike animal models, in which the tumours can easily be removed to evaluate the efficacy of treatment, human serial biopsies might not be a particularly practical or desirable approach. Therefore, considerable research effort is being made in this area to establish surrogate markers, such as measurement of protein levels in serum or urine, and to develop a noninvasive strategy.

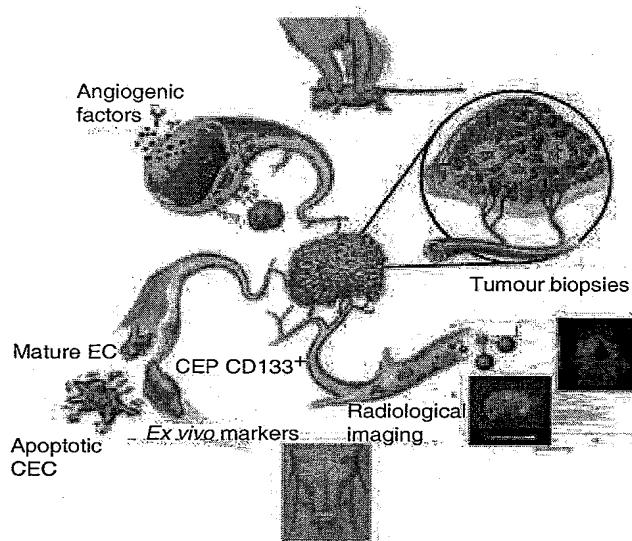


Figure 4. Surrogate markers of tumour angiogenesis activity. The use of antiangiogenesis therapy has created an urgent need to develop markers of activity. Several avenues are currently being investigated, such as tumour biopsy analysis, radiological imaging (chemotherapy, positron emission tomography, MRI) and levels of angiogenic factors (in serum, plasma, urine) or isolated blood cells (endothelial cells).

Published with permission of DAVIS DW, MCCONKEY DJ, ABRUZZESE JL, HERBST RS, *Br. J. Cancer* (2003) 89:8-14 [132].
CEC: Circulating EC; EC: Endothelial cell.

Several markers of efficacy have been investigated with mitigated outcomes. Microvessel density was also suggested as a means to predict response to angiogenesis inhibitors [124]. Tumours are stained for markers such as CD34 or CD31 and the number of blood vessels are quantified by scanning the tumour areas for vascularity [125]. Imaging studies such as computed tomography and dynamic contrast-enhanced magnetic resonance imaging [126] may be helpful in assessing the efficacy of treatment and potentially the appropriate dose of angiogenic agents. Further research is ongoing to examine if other potential biological markers such as receptors in the tumour endothelial cell can be relevant. Identifying biological markers is undeniably of great interest to monitor the efficacy of these new agents, but they may also prove to be valuable in selecting patients who may benefit from targeted therapy.

6.5.3 Resistance to antiangiogenic therapies: a rising issue

In late-stage tumours, phenotypic resistance to VEGFR-2 blockade emerges, as tumours grow again during treatment, after an initial period of growth suppression due to angiogenic agents. This resistance to VEGF inhibitors involves reactivation of tumour angiogenesis and certainly the involvement of other pro-angiogenic factors. The nature of these other

pro-angiogenic signals, as well as the exact mechanisms responsible for tumour revascularisation and regrowth in the evasion phase, remain largely unknown. An example that might illustrate this phenomenon of resistance is the negative results of a study of bevacizumab in late-stage mBC patients. In this Phase III trial, the addition of bevacizumab to capecitabine failed to achieve significant improvement in PFS or OS in previously treated mBC patients [57]. Considering the clinical benefits of bevacizumab in earlier-line treatment of CRC, the results of this trial were surprising, but suggest that the administration of angiogenic drugs at earlier stages of the disease might be more effective and beneficial. It is possible to conceive that as the disease progresses, redundant pathways might be implicated, with VEGFA being replaced by other factors including VEGF-unrelated or other members of the VEGF gene family such as VEGFC and VEGFD.

Work by Yu and colleagues has shown that tumour cells harbouring genetic alterations of the p53 gene, a tumour suppressor gene inactivated in most human cancers, display a lower apoptosis rate under hypoxic conditions, which might reduce their reliance on vascular supply and thereby their responsiveness to antiangiogenic therapy [127]. Therefore, the selection and overgrowth of tumour-variant cells that are hypoxic resistant and less dependent on angiogenesis could explain the resistance to antiangiogenic drugs. Although endothelial cells have long been assumed to be genetically stable, they might under some circumstances harbour genetic abnormalities and acquire resistance [128].

7. Expert opinion

After 30 years of intense clinical studies, antiangiogenesis therapy has finally found its place in mainstream therapy of human malignancies. Although the traditional lines of treatment such as chemotherapy, radiation therapy and surgery still remain of significant importance, the emergence of anti-VEGF therapeutic modalities has brought hope for both patients and physicians. Indeed, antiangiogenesis therapy has proved efficacious in the treatment of breast, colon, renal and lung cancers and data from ongoing clinical trials clearly indicate that soon other tumour types will also benefit from these agents. Rarely has the world of cancer research seen such enthusiasm as that surrounding the approval of antiangiogenesis drugs. Bevacizumab was the first monoclonal antibody against VEGF to be approved, and the recent approval of sunitinib and sorafenib, two VEGFR TKIs, in GIST and RCC, have widened our treatment options and established the therapeutic benefits of antiangiogenic agents in our current armamentarium. Incontestably, it is an exciting time for physicians to participate in clinical trials involving antiangiogenic agents and to witness hopefully the approval of more drugs in the near future.

Several critical issues have emerged from clinical trials, and it is our biggest challenge for the next decades to appropriately address these concerns. Identifying the most effective

therapeutic strategy is definitely a major issue. As the extensive complexity of the molecular mechanisms involved in tumourigenesis is being unveiled, it seems clear that a combinatorial approach strategy will lead to better clinical outcomes. Two strategies may be followed in choosing targeted therapeutic agents: a vertical approach aimed at multiple targets within the same pathway (e.g., anti-VEGF combined with VEGFR TKI) or a horizontal approach whereby multiple pathways (e.g., VEGFR and EGFR) are targeted to prevent the generation of escape variants. Although the monoclonal antibodies and TKIs reviewed in this manuscript interfere with angiogenesis, results from clinical trials have clearly demonstrated that these two classes of agents bear an individual spectrum of activity; hence, there is a need to determine the most effective agent based on tumour type. Additional clinical trials need to be designed with the primary goal of identifying the correct dosing, setting and right combination therapy to ensure that patients will fully benefit from these new agents. Furthermore, the lack of reliable surrogate markers of activity has hindered the clinical evaluation of the efficacy of these new drugs. Additional experimental models and well-designed clinical trials will surely help us identify

surrogate markers of tumour angiogenesis and selection markers. Understanding targeted therapy resistance and assessing the potential cumulative toxicities are among the end points that need to be considered in future trials.

Although common mechanisms are dysregulated in the vast majority of human malignancies, individual genetic variability might also play a significant role in responsiveness to targeted therapy. Will the future of cancer treatment include a more personalised approach based on the genetic background of each patient? Despite many unanswered questions, antiangiogenic therapy is efficacious and needs to become a standard therapeutic option. It is our responsibility to carry out well-planned clinical trials as we seek to eliminate cancer and suffering. This review highlights some of the newest agents against vascular targets. The hope is that their use remains high and their ability to change the face of cancer therapy is potent.

Acknowledgement

The author thanks Amgen for funding IntraMed, New York, for editorial support in the preparation and styling of this manuscript.

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